Poster # 52

Quorum Sensing (QS) Molecules Released by Gram Negative Bacteria induce tubular injury interacting with lipopolysaccharide (LPS): a new potential mechanism of sepsis-associated AKI

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Background

Quorum sensing (QS) are small diffusible signal molecules adopted by Gram negative and Gram positive bacteria for intercellular communication. QS are a system of transcriptional regulation dependent on cell density and formed by 2 elements: the signal molecule (usually an acyl-homoserine lactone) able to be internalized in the cytoplasm of neighboring cells and the transcriptional activator.

QS play a crucial role in biofilm formation and are known to bind the lipid A fraction of LPS.

Recent studies suggested that QS can also interact with eukaryotic cells exerting immunomodulatory effects and playing a key role in urinary tract infection.

Aim of the study

The aim of this study was to evaluate the potential role of QS in sepsis-associated AKI by studying their biological effects on human kidney tubular epithelial cells (TEC).

Methods

Human TEC were isolated from kidneys of patients subjected to nephrectomy. QS from P. aeruginosa were purchased by Sigma Aldrich. In selected experiments supernatants of QS-negative mutants of P. aeruginosa were used on TEC.
We evaluated QS-induced: cytotoxicity (XTT), apoptosis (TUNEL, caspase-3, -8, 9 activities), alteration of cell polarity (trans-epithelial electrical resistance, TEER), ROS production, NGAL mRNA/protein expression, *in vitro* leukocyte adhesion and FACS or immunofluorescence analysis of molecules typical of fully differentiated TEC (ZO-1, megalin, AQP-2, E-cadherin) or involved in inflammation (ICAM-1,CD40).

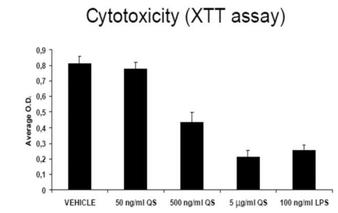

Fig. 1: Dose-dependent (50-5000 ng/ml) cytotoxic effect (XTT assay) exerted by QS on human tubular epithelial cells. LPS (100 ng/ml) was used as positive control.

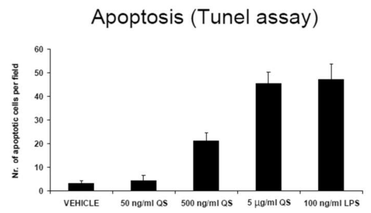

Fig. 2: Dose-dependent (50-5000 ng/ml) pro-apoptotic effect (TUNEL assay detecting DNA fragmentation) exerted by QS on human tubular epithelial cells. LPS (100 ng/ml) was used as positive control.

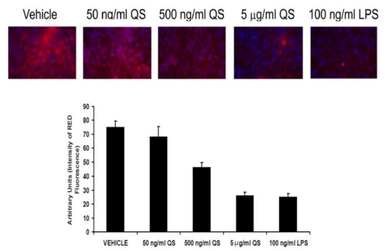

Fig. 3: Dose-dependent (50 - 5000 ng / ml) alteration of mito-chondrial membrane potential (Mytotracker red fluorescence) induced by QS on human tubular epithelial cells. LPS (100 ng/ml) was used as positive control.

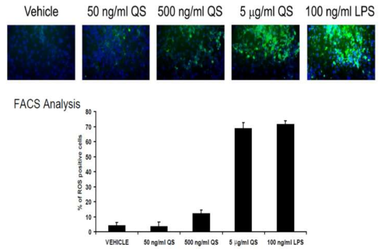

Fig. 4: Dose-dependent (50-5000 ng/ml) induction of ROS expression induced by QS on human tubular epithelial cells. LPS (100 ng/ml) was used as positive control.

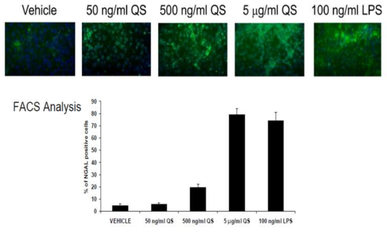

Fig. 5: Dose-dependent (50-5000 ng/ml) increase of NGAL expression (immunofluoresence and FACS analysis) induced by QS on human tubular epithelial cells. LPS (100 ng/ml) was used as positive control.

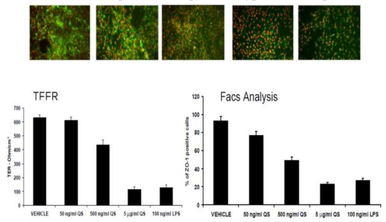

Fig. 6: Dose-dependent (50-5000 ng/ml) decrease of the tight junction molecule ZO-1 expression (immunofluorescence and FACS analysis) and loss of cell polarity (TEER) induced by QS on human tubular epithelial cells. LPS (100 ng/ml) was used as positive control.

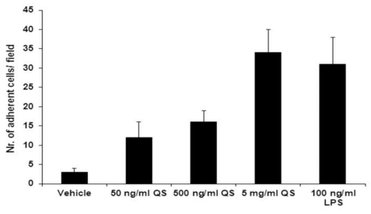

Fig. 7: Dose-dependent (50-5000 ng/ml) increase of leukocyte adhesion induced by QS on human tubular epithelial cells. LPS (100 ng/ml) was used as positive control.

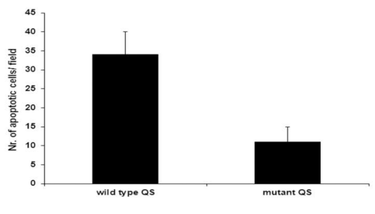

Fig. 8: Evaluation of cytotoxicity (XTT assay) and apoptosis (TUNEL assay) induced by supernatants of wild type or QS-mutant P. aerugionosa on human tubular epithelial cells.

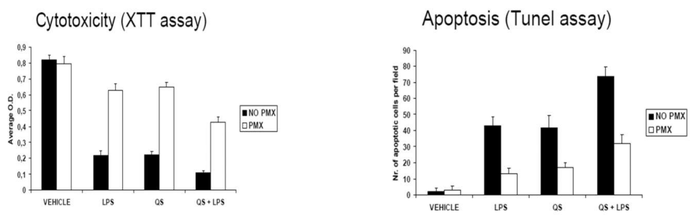

Fig. 9: Evaluation of cytotoxicity (XTT assay) and apoptosis (TUNEL assay) showing the synergistic detrimental effect of QS and LPS on tubular epithelial cells and the potential protection exerted by PMX-B.

Results

QS exerted a dose-dependent cytotoxic (XTT assay in Fig. 1) and pro-apoptotic effect (TUNEL assay in Fig. 2) on TEC inducing Fas and caspase activation. The percentage of tubular cell apoptosis was similar to that observed in presence of LPS. The pro-apoptotic effect of QS on tubular cells seems to be mediated by an alteration of mitochondrial membrane potential (Mytotracker in Fig. 3), increase of ROS production (Fig. 4) and NGAL release (Fig. 5). In addition, QS induced de-differentiation in TEC as assessed by loss of cell polarity with a significant decrease of TEER and of expression of ZO-1 (Fig. 6), megalin and AQP-2. QS induced a pro-inflammatory effect on TEC increasing leukocyte adhesion (Fig. 7) and surface expression of ICAM-1 and CD40. A similar pro-apoptotic effect on TEC was observed using supernatants produced from wild type P. aeruginosa, but not from QS negative mutants (Fig. 8). The cytotoxic and pro-apoptotic effect of QS on TEC was enhanced by co-incubation with LPS. By contrast, QS-induced TEC injury was significantly decreased after adsorption *in vitro* with polymyxin-B (PMX-B) (Fig. 9). Preliminary results obtained using HPLC-MS revealed that QS are detectable in the bloodstream of patients with Gram-negative sepsis. In addition to the interaction with the lipid A fraction of LPS, QS seem to have a direct hydrophobic binding to PMX-B.

Conclusions

QS derived from Gram negative bacteria may induce different detrimental effects on TEC such as cytotoxicity, apoptosis, mitochondrial dysfunction, ROS generation, NGAL release, leukocyte adhesion and de-differentiation with loss of polarity and of expression of the tight junction protein ZO-1. QS may enhance the pro-apoptotic activity of LPS. By contrast, PMX-B may have a protective role by binding QS directly or through interaction with LPS.

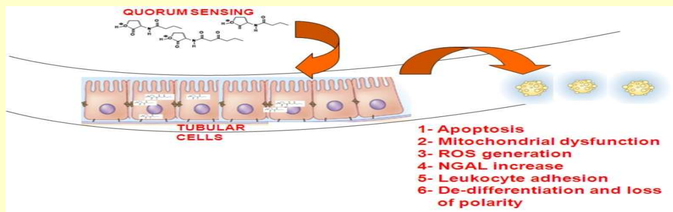

Fig. 10: Review of the detrimental biological effects exerted by QS on human tubular epithelial cells and the potential correlation with sepsis-associated AKI.